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METABOLISM OF CYCLOHEXYL-ISOPROPYLAMINES IN MAN IN COMPARISON WITH AMPHETAMINES*)

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INTRODUCTION

The metabolism of amphetamines and their derivatives with substituents in the propyl side chain have been studied extensively over the past 10 years. (1, 3, 4, 6, 7, 8, 9, 10, 14, 15, 20). The possible routes for metabolism are parahydroxylation, α-C-oxidation (deamination and dealkylation) and N-oxidation. The lipid-solubility plays an important role in directing routes of the total elimination, because a high lipid-solubility results in a less renal clearance, a great volume of distribution, a longer life time in the body and consequently more extensive metabolism. Weak lipophilic properties cause a high renal excretion of the unchanged compound and subsequently low degree of metabolism, such as can be observed with the ephedrines (15, 20).

In general, ring substitution results in an increase of lipid-solubility, accompanied by a greater metabolic clearance. This has been observed for fenfluramine (4), chloro-amphetamine (7) and methoxyphenamine (19). Relatively little is known about hydrogen substituted amphetamine, the cyclohexylisopropylamine, and its N-alkyl substituted derivatives, ('saturated amphetamines'), while N-methylcyclohexylisopropylamine, propylhexedrine, is in use as an anti-obesity drug.

The aim of the investigation was to study the influence of the hydrogen substitution on the metabolic pattern. The metabolic fate of the saturated amphetamines in man was compared with the known metabolic pathways of amphetamines and ephedrines.

EXPERIMENTAL

Synthesis: Amphetamine and derivatives (+), (−) were hydrogenated in glacial acetic acid with PtO_2/H_2 (3 atm) in a Parr apparatus. The S (+) amphetamine was converted into S (−) cyclohexylisopropylamine. Ephedrines were treated in the same way to corresponding cyclohexylisopropanolamines. N-cycloalkylamphetamines were prepared by boiling (+) amphetamine in excess of cyclopentanon, cyclohexanon and cycloheptanon,

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resp. with Mg_2SO_4, followed by reduction with sodiumborohydride. All reactions were followed by means of combined GC-MS (LKB 9000).

Gas chromatography: H P 402 gas chromatograph with flame ionisation detector. Columns: 20% Apiezon M, 5% KOH on Gaschrom Q 60—80 mesh and 3% OV 17 on gaschrom Q 60—80-mesh. Amphetamines, ephedrines and its saturated analogues cannot be separated at these columns from each other. The hydroxy metabolites have a retention time of 2.20 times the retention time of the parent compound. After hydrolysis of the urine with 6N HCl, no conjugated amphetamines could be detected.

Mass spectrometry: L.K.B. 9000 combined gas chromatograph- mass spectrometer, equipped with a column of 20% Apiezon-5% KOH on gaschrom Q 60—80 m. Column temperature 160°, separator 200°, ion source 290°, trapcurrent 60 μA, accelerating voltage 3,5 kV, ionisation voltage 20—70 eV. The hydroxy metabolites of the N-cyclohexylamphetamine could be separated at the column and the position of the OH group could be identified. With cyclohexylisopropylamines, the separation of the hydroxy metabolites was too poor. Full details will be described elsewhere (19).

Metabolism: 30—60 mg of the HCl salts of the compounds were taken orally by healthy human subjects (25—30 years, 70—85 kg). The urine was kept acidic (pH 5 $\pm$0.3) by the daily intake 4 $\times$ 2 grams of ammonium chloride (4 tablets of 0.5 g 4 dd). Each urine sample was collected, made alkaline, extracted with ether and analysed with the GLC as described before (14, 15). Renal excretion rate and cumulative renal excretion was plotted for each urine sample versus the time after administration (see Fig. 1—3) Dissociation constants (Ks) characterizing binding to microsomal enzymes were measured as described by VREE and HENDERSON (17, 18).

RESULTS AND DISCUSSION

A. *Excretion of the unchanged compound*

After ingestion of cyclohexylisopropylamine or an N-alkyl substituted derivative by man, a very small amount of the parent compound was excreted unchanged in the urine.

TABLE 1

Excretion of unchanged amphetamines and cyclohexylisopropylamines in the urine of man

Compound	% dose excreted unchanged and half life time of elimination			
	S configuration	$T^1/_2$	R configuration	$T^1/_2$
Cyclohexylisopropylamine	7 %	3 hr	6 %	3 hr
.. N-methyl	7	3	7	5
.. N-ethyl	4	3.5	4	3
.. N-isopropyl	1	0.5	3	1
Amphetamine	70	7	90	8
.. N-methyl	65	7	80	7
.. N-ethyl	40	5	90	7
.. N-isopropyl	10	3	85	7

In Table 1 the amounts excreted are given as well as the percentages obtained with the corresponding amphetamines (Fig. 1).

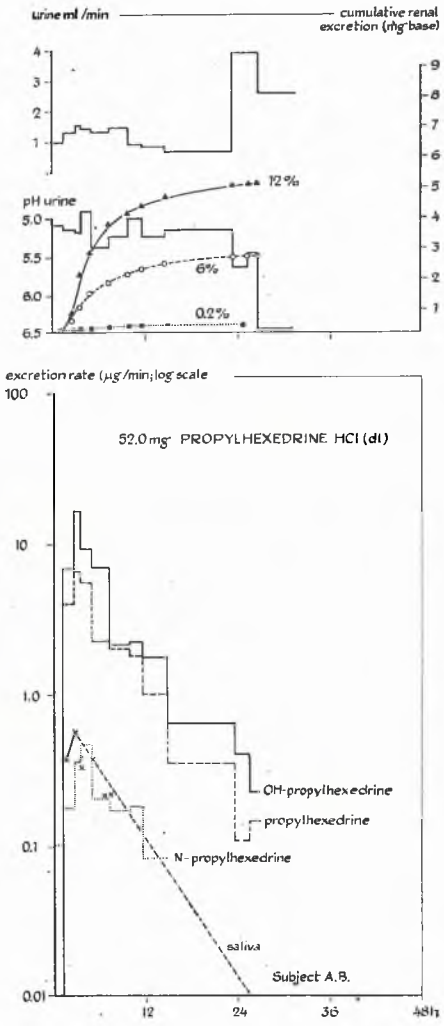

Fig. 1. Renal excretion, urinary pH, average urine production, cumulative renal excretion of N-methylcyclohexylisopropylamine (propylhexedrine) and its metabolites cyclohexylisopropylamine and cyclohexanolisopropylamine. The metabolic processes are very fast, the renal excretion of the metabolites reached a maximum after 2—3 hours.

From the table it can be derived that as the saturated amphetamines are concerned there is no striking difference in metabolic behaviour between the various derivatives and between the different stereochemical configurations of those derivatives. All compounds are very well metabolized and the renal excretion of the unchanged compound

is of minor importance .This is in contrast to the amphetamine series, where substantial amounts of unchanged compounds are excreted. For the derivatives with the S configuration the proportional excretion of the unchanged compound decreases with increasing alkyl substituent. However, this excretion is constant for the R configuration (14).

TABLE 2

Percent dose excreted as ring hydroxylated metabolites into the urine of man

Compound	Configuration		
	(—) S	(+) R	S/R
Cyclohexylisopropylamine	0	0	
.. N-methyl	6.0	10	0.60
.. N-ethyl	37	55	0.67
.. N-isopropyl	55	85	0.64

The detection system used, makes it possible to detect the parahydroxylated metabolites of the saturated amphetamine in contrast to parahydroxyamphetamine. In spite of this possibility, no trace of hydroxylated metabolite of cyclohexylisopropylamine was found. It must be concluded that the deamination is an extremely fast metabolic route in the metabolism of this parent compound. Most likely deamination and ring hydroxylation are competitive pathways for elimination. With the amphetamines α-C-oxidation and renal excretion are the two metabolic routes.

B. *Hydroxy metabolites*

In comparison with the amphetamine metabolism, the most striking observation in the metabolism of saturated amphetamines is the hydroxylation of the cyclohexyl ring. The hydroxylation is prevailing when the seize of the substituent increases, concomitantly with increase of the lipid-solubility. The degree of hydroxylation also depends upon the stereochemical configuration, but the ratio of hydroxylation of the R and S configuration is constant (Fig. 2). The results are summarized in Table 2.

TABLE 3

Aromatic hydroxylation of amphetamine antipodes in various species (after SMITH and DRING, 12)

Species	% dose excreted as *p*-hydroxyamphetamine		
	S (+)	R (—)	R, S (+, —)
Man	1.1	3.9	2.8
Guinea pig	0	1	
Mouse	14	17	
Rat	48	63	60

The differences in percentage of the hydroxy metabolites in the R and S configuration might be explained with the aid of the competitive metabolic pathways, on the understanding that the S-compound is more susceptible to α-C-oxidation than the R-form.

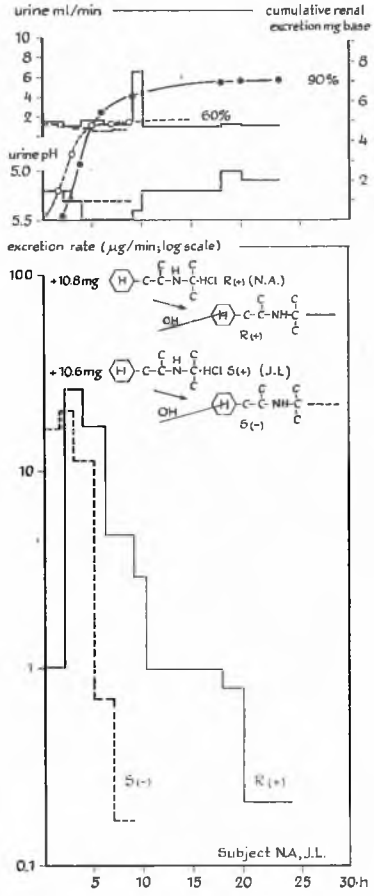

Fig. 2. Difference in renal excretion rate between the R (+) and S (—) configuration of the metabolite N-isopropyl-cyclohexanolisopropylamine. The R (+) configuration ($T^1/_2$ = 4 hr) is much slower metabolized (α-C-oxidation) than the S (—) configuration. The equal maximum renal excretion rate of both metabolites indicates that the rate of hydroxylation is independent of the stereochemical configuration of the side chain.

Deamination is so fast that N-dealkylated metabolites are hardly excreted unchanged in the urine (1—5%). As reported earlier, for amphetamines both reactions were extremely dependent upon the R- and S configuration (14). The same behaviour can be found with saturated amphetamines, the effect in some way is masked by the hydroxylation. DRING (7) showed for some species the amounts of parahydroxylation of S- and R amphetamine, and he found the same differences in hydroxylated products, due to the difference in deamination of the amphetamine (Table 3).

C. *The hydroxylating enzyme system*

Considering the metabolism of cyclohexylisopropylamines and amphetamines the question arises whether one enzyme performs both α-C-oxidation and ring hydroxylation, or different enzymes, or different active sites of one enzyme system are responsible for the conversion. What is the reason why with increasing size of the N-alkyl substituent with saturated amphetamines the ring hydroxylation increases while with amphetamines the α-C-oxidation increases. An explanation may be that with increasing the seize of t substituent, the lipid-solubility increases too and that 'a certain part' of the enzyme can be reached while with amphetamines the nature of the C—H bond and therefore the rate of the α-C-oxidation is altered. This may be acceptable when one assumes that for all derivatives the rate of aromatic hydroxylation is low. The lipid-solubilities were measured, but it turned out that the corresponding derivatives of saturated and non saturated amphetamines have a fixed ratio of their partition coefficient (Table 4).

TABLE 4

pKa values and partition coefficients of amphetamine, cyclohexylisopropylamine and derivatives

Compound	pKa	TPC_{hept}	TPC_{chlor}	APC_{hept}	APC_{chlor}
Amphetamine	9.90	1.88	146	0.005	0.48
.. N-methyl	10.10	5.14	565	0.015	1.10
.. N-ethyl	10.23	38.6	1790	0.060	2.67
.. N-isopropyl	10.14	117	4460	0.21	8.09
Cyclohexylisopropylamine	10.50	19.0	770	0.015	0.61
.. N-methyl	10.60	92.0	1860	0.058	1.17
.. N-ethyl	10.80	550	8350	0.22	3.34
.. N-isopropyl	10.60	1180	12900	0.74	8.10

TPC – true partion coefficient
APC – apparent partition coefficient
hept – system heptane-water (Teorell buffer, pH 7.4)
chlor – system chloroform-water (Teorell buffer, pH 7.4)

The different saturated amphetamines are about three times more lipid-soluble than the corresponding amphetamines. This difference in solubility does not explain the different enzymatic attack at the molecule. The difference between the two ring systems is the nature of the C-H bond that is attacked, aliphatic and aromatic, and the 'shape' of the molecule.

The phenyl ring is stabilized by its resonance energy of 36 kcal/mol. If one enzyme is postulated for hydroxylation of both phenyl ring and cyclohexyl ring than the resonance energy may act as a threshold. Due to its high activation energy of the hydroxylation of the phenyl the α-C-oxidation get a chance to occur as an alternative metabolic pathway. The cyclohexyl ring behaves like an aliphatic chain, in which the most outstanding C-H bond with the lowest energy is hydroxylated. It may be that different enzyme systems (mechanisms) are metabolizing these amphetamines. The compound ferrocenylisopropylamine exerts to be an excellent inhibitor of the metabolism of amphetamines in the rat (17, 18). Ferrocenylisopropylamine binds very strongly to the cytochrome P-450 (Table 5)

and in this way inhibits the α-C-oxidation. But, even when the ferrocenylisopropylamine was added in an excess of 50 times the concentration of saturated isopropylamphetamine in rats, the compound failed to inhibit the metabolism of saturated isopropylamphetamine. From this observation it must be concluded that the hydroxylation of the cyclohexyl ring is mediated by an other mechanism than α-C-oxidation. The binding affinities of the amphetamines and its saturated analogues are found to be of the same magnitude rats. This means that the difference in structure of the phenyl and cyclohexyl ring has no influence at the interaction drug-cytochrome P-450. In Table 5 the binding affinities of the amphetamines and related compounds are given.

TABLE 5

Binding affinities of amphetamines, cyclohexylisopropylamines and related compounds to hepatic cytochrome P-450 of the rat

Compound	Ks value molar	Type
Amphetamine (S)	5.0×10^{-3}	I
Amphetamine (R)	4.5×10^{-3}	I
N-isopropylamphetamine (S)	5.0×10^{-3}	I
N-isopropylamphetamine (R)	4.1×10^{-3}	I
Cyclohexylisopropylamine (S)	4.0×10^{-3}	I
.. N-isopropyl (S)	5.0×10^{-3}	I
Benzphetamine (S)	5.5×10^{-4}	I
Ferrocenylisopropylamine	1.4×10^{-6}	II
.. N-isopropyl	5.0×10^{-6}	II
DPEA	1.4×10^{-6}	I
Imipramine	2.2×10^{-7}	I (2)
Desmethylimipramine	2.7×10^{-6}	I (2)
Amitriptyline	2.5×10^{-7}	I (2)

LEWANDER (11) reported the inhibition of both parahydroxylation (95%) and deamination (50%) of amphetamine by desmethylimipramine. The Ks value of imipramine and desmethylimipramine is of the same magnitude as that of ferrocenylisopropylamine (2) Table 5). This finding supports the idea that if the enzyme is blocked by these strong bounded compounds, all possible metabolic pathways that can be performed by the occupied enzyme entity, are blocked. The same results were obtained by inhibition of the amphetamine metabolism by the compound DPEA (10).

It is postulated by BRODIE (5) and UDENFRIEND (13) that the cytochrome P-450 hydroxylates the aromatic nucleus by the epoxide mechanism. Such a mechanism cannot be involved for the hydroxylation of the cycloalkyl ring.

What are the requirements for an enzymatic hydroxylation of cycloalkyl rings? Is the activation energy of the rupture of the C-H bond the dominating factor or are there also any steric requirements? Is it possible to replace the cyclohexyl ring by a cyclopentyl ring without alteration of metabolic pathways. This should be possible if only the activation energy mentioned is determinant for the hydroxylation.

The compound N-methylcyclopentylisopropylamine, cyclopentamine, is demethylated and excreted into the urine like methylamphetamine (Fig. 3). In man no ring hydroxylation could be observed. The cyclopentyl ring behaves like a phenyl ring, both ring systems appeared to have strong influence at the metabolic routes. The cyclopentyl ring is a planar ring system, chemically stable and without ring tension. It must be concluded that the hydroxylating enzyme can easily attack molecules with a certain flexibility in the C-H bond that can be attacked. This is the case with aliphatic carbon chains and alicyclic ri[r] systems. A cyclopentyl ring is planar and rigid and the same holds for the phenyl ring.

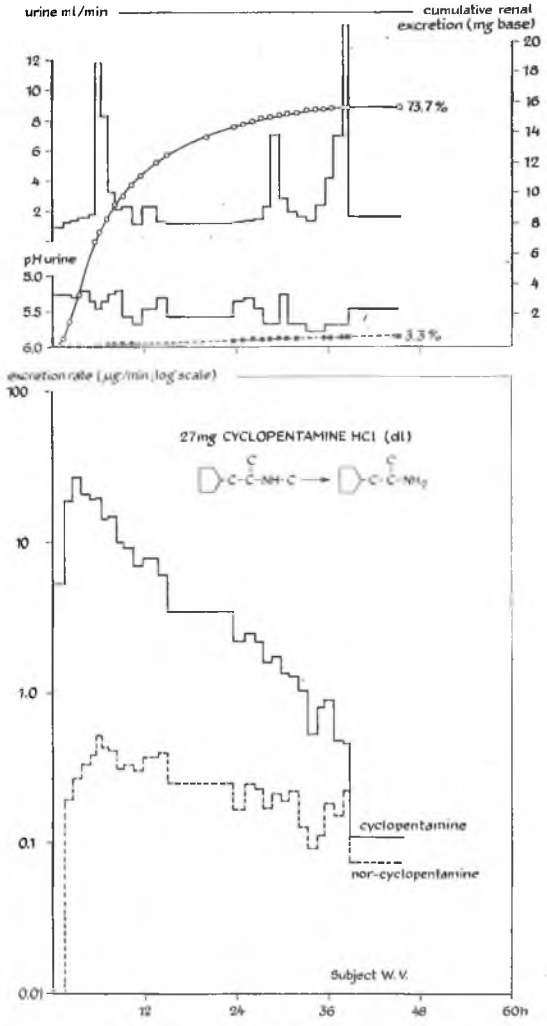

Fig. 3. Renal excretion rate, cumulative renal excretion, urinary pH and average urine production of cyclopentamine. The compound is excreted like methylamphetamine. Cyclopentamine: $T^1/_2$ = 7 hr, 70 % totally excreted unchanged, 5 % α-C-oxidation. Methylamphetamine: $T^1/_2$ = 7 hr, 70 % excreted and 10 % α-C-oxidation. Even the relationship dose mg HCl = µg/min max. excretion rate exists.

D. *N-cycloalkyl substituted amphetamines*

With amphetamines the main metabolic pathways in man is the α-C-oxidation and with the saturated amphetamines α-C-oxidation competes with ring hydroxylation. Also the 'flexibility' of the ring is demonstrated. In N-cycloalkyl substituted amphetamines, all those competitive pathways are possible. In order to get information about relative rate constants of α-C-oxidation and alicyclic ring hydroxylation, the following compounds were investigated: N-cyclopentyl-, N-cyclohexyl-, and N-cycloheptylamphetamine (S+).

In this series the tertiary C-H bond of the cycloalkyl group has the same outstanding position as the corresponding tertiary C-H in the amphetamine moiety. The rate of formation of the metabolite amphetamine gives an indication about the sterical hindrance of the bulky ring system when the compound is attacked by the cytochrome P-450 for α-C-oxidation. In Table 6 compounds are compared with 2 tertiary C-H bonds available for a α-C-oxidation by the cytochrome.

TABLE 6

Metabolism of N-cycloalkyl substituted amphetamines. Comparison of the availability of the tertiary C-H- bond of the substituent for attack by the cytochrome P-450

Compound	% dose excreted unchanged	% dose excreted as amphetamine	% dose excreted as ring hydroxylated metabolite of N-cycloalkylamph.
(+) isopropylamphetamine	10	40	0
(+) N-cyclopentylamphetamine	3	30	0
(+) N-cyclohexylamphetamine	1	5	18
(+) N-cycloheptylamphetamine	0	5	0

From the table it can be derived that all possible metabolic pathways do occur.

Cyclopentylamphetamine. The amount amphetamine formed and excreted in the urine indicated that the tertiary C-H of the cyclopentyl group can be attacked very easily by the cytochrome P-450. It is almost the same situation as observed with isopropylamphetamine. The ring system exhibits no or very slight sterical hindrance in the interaction with the enzyme. The tertiary C-H binding can be attacked by the cytochrome when this group is adjacent to the nitrogen atom. However, in N-methylcyclopentylisopropylamine, this particular C-H group is not attacked at all (see Fig. 3). This means that the α- C-oxidation only occurs in the aliphatic side chain and that the nitrogen atom must be a 'target' atom for the enzyme. Therefore it is also less probable that α-C-oxidation is responsible for hydroxylation of alicyclic ring systems.

Cyclohexylamphetamine: The low amount of amphetamine formed as metabolite from N-cyclohexylamphetamine indicates that the C-H bond is still accessible for attack by the cytochrome, but also that other metabolic pathways reduce the significance of this contribution to the total elimination. With N-cyclohexylamphetamine, the main metabolite excreted in the urine is N-cyclohexanolamphetamine. The total amount was about 18%, and the OH group was distributed over the ring as follows: 0.8% C2, 2.2% C3 and 15% C4. The situation is the same as observed with the saturated amphetamines. Again, α-C-oxidation and alicyclic ring hydroxylation are the competitive metabolic pathways.

Cycloheptylamphetamine: As compared to the N-cyclohexylamphetamine, the seize of the ring has no influence at the rate of formation of amphetamine. It was not possible to detect alicyclic ring hydroxylated metabolites. The metabolic routes of this compound are not clear.

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